RHEUMATOLOGY

Rheumatology 2010;49:1683–1693
doi:10.1093/rheumatology/keq116
Advance Access publication 12 May 2010

Original article

Efficacy and safety of various repeat treatment dosing regimens of rituximab in patients with active rheumatoid arthritis: results of a Phase III randomized study (MIRROR)

Andrea Rubbert-Roth[1], Paul P. Tak[2], Cristiano Zerbini[3], Jean-Luc Tremblay[4], Luis Carreño[5], Gillian Armstrong[6], Neil Collinson[6] and Tim M. Shaw[6] on behalf of the MIRROR Trial Investigators*

Abstract

Objective. To evaluate the efficacy and safety of three dosing and repeat treatment regimens of rituximab (RTX) plus MTX in patients with active RA.

Methods. Patients with active RA despite stable MTX (10–25 mg/week) were randomly assigned to one of the three treatment regimens comprising two courses of RTX given 24 weeks apart: 2 × 500 and 2 × 500 mg; 2 × 500 and 2 × 1000 mg (dose escalation); and 2 × 1000 and 2 × 1000 mg. The primary endpoint was proportion of patients achieving ACR20 at Week 48.

Results. At Week 48, ACR20 responses were not statistically significantly different between the dose regimens. Compared with RTX 2 × 500 mg ($n = 134$) or dose escalation ($n = 119$), ACR and European League Against Rheumatism (EULAR) outcomes in the RTX 2 × 1000 mg group ($n = 93$) were consistently higher, with significantly more patients achieving EULAR responses ($P = 0.0495$). At Week 48, rituximab 2 × 1000 mg was associated with a higher proportion of patients who, following retreatment, maintained or improved their Week 24 responses. Dose escalation from 2 × 500 to 2 × 1000 mg did not appear to be associated with improved outcomes compared with continual 2 × 500 mg. All RTX regimens demonstrated comparable safety.

Conclusions. RTX 2 × 500 and 2 × 1000 mg could not be clearly differentiated, although some efficacy outcomes suggest improved outcomes in the rituximab 2 × 1000 mg group. Retreatment from Week 24 resulted in a sustained suppression of disease activity through to Week 48.

Trial registration. ClinicalTrials.gov, http://clinicaltrials.gov/, NCT00422383.

Key words: Rituximab, Rheumatoid arthritis, Repeat treatment, B-cell depletion, Phase III.

CLINICAL SCIENCE

[1]Department of Internal Medicine, University of Cologne, Cologne, Germany, [2]Department of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, [3]Department of Rheumatology, Hospital Heliópolis, São Paulo, Brazil, [4]Department of Rheumatology, Centre Hospitalier Régional de Trois-Rivières, Trois-Rivières, QC, Canada, [5]Department of Rheumatology, Hospital Gregorio Marañón, Madrid, Spain and [6]Roche Products Ltd, Welwyn Garden City, UK.

Submitted 1 October 2009; revised version accepted 15 March 2010.

Correspondence to: Andrea Rubbert-Roth, Klinikum der Universität zu Köln, Klinik für Innere Medizin I, Josef-Stelzmann-Strasse 9, 50931 Köln, Germany. E-mail: andrea.rubbert@medizin.uni-koeln.de

*See Appendix 1 for the MIRROR Trial Group investigators.

Introduction

Rituximab (RTX), an mAb that selectively targets and depletes CD20$^+$ B cells, has demonstrated significant efficacy and a favourable safety profile in clinical trials conducted in patients with active RA [1, 2]. RTX 2 × 1000 mg in combination with MTX resulted in a significant clinical and radiographical benefit in patients with an inadequate response or intolerance to TNF inhibitors [3], and this dose or a lower dose of 2 × 500 mg resulted in significant improvements in disease activity in

patients with an inadequate response to non-biological DMARDs [4].

Consequently, questions remain, not only regarding the most appropriate dose of RTX, but also how and when patients should receive further courses. In long-term observational studies, patients who had an initial response to RTX were allowed further courses no more frequently than every 16 weeks if they had active disease (defined by at least eight swollen and eight tender joints) [5], with the decision to retreat also being at the discretion of the treating physician. As a consequence, at an individual patient level, repeat treatment times were highly variable, with clear evidence of returning disease between treatment courses. While defining a fixed repeat treatment schedule suitable for all patients may not be appropriate, it would, however, be desirable to retreat patients before a significant clinical flare occurs.

Further, the benefit of repeat treatment in patients in whom an initial response was not achieved has not been established and requires further investigation. Similarly, data on any effect of dose used for such repeat treatments may provide clinically relevant information.

Therefore, the present study was designed to determine if initiating treatment with RTX 2×500 mg followed by a repeat treatment at 24 weeks with 2×500 mg was different from repeat treatment with a higher dose of 2×1000 mg. The study was also designed to compare the efficacy and safety of RTX 2×500 and 2×1000 mg over 48 weeks with a fixed repeat treatment at Week 24.

Methods

Study design

This study was a multicentre, randomized, double-blind, Phase III trial conducted as part of the clinical development programme for RTX in patients with an inadequate response to disease modifying therapies. The study was conducted at 81 centres in 18 countries in patients with active RA who had an inadequate clinical response to MTX therapy. The overall study design is shown in Fig. 1. Patients were randomly assigned to three treatment groups: initial treatment with RTX 2×500 mg with a repeat course at Week 24 also of 2×500 mg; dose escalation (initially RTX 2×500 mg, with 2×1000 mg on retreatment); or initial treatment with rituximab 2×1000 mg and retreatment with 2×1000 mg. All RTX infusions were preceded by intravenous methylprednisolone 100 mg.

The pharmacokinetic profile of RTX shows that by 16–24 weeks, drug levels are below the level of detection and there is evidence of gradual repletion of peripheral $CD19^+$ B cells [6], which in some patients may precede recurrence of active disease. Further, evidence suggests that even low circulating CD19 levels may be associated with poor response or returning disease [7]. Retreatment at 24 weeks, therefore, represents a reasonable time at which to retreat.

Patients were randomly allocated using an interactive voice response system; the randomization was stratified by region, RF seropositivity and prior biological use. Although all patients were randomly assigned to RTX-containing regimens, allocation to dose and repeat treatment regimen was blinded. The sponsor, investigators and patients were blinded to the treatment allocation up to the time of the Week 48 analysis. Treatment assignments were unblinded to the sponsor at this time for the purpose of the data analysis.

Stable doses of MTX (10–25 mg/week) were maintained throughout the study period. Permitted co-medications included folic acid (5 mg/week) NSAIDs and oral glucocorticoids ($\leqslant$10 mg/day). IA glucocorticoid injections were restricted to not more than one joint per 24-week period. Use of additional non-biological and biological DMARDs was strictly prohibited.

The study was performed in accordance with the Declaration of Helsinki. All participating sites received approval from their governing institutional review board (or equivalent) and all patients provided written informed consent. The study is registered with ClinicalTrials.gov: NCT00422383.

Patients

Inclusion criteria included a diagnosis of RA (according to the revised 1987 ACR criteria for the classification of RA) for at least 6 months with active disease, despite MTX at 10–25 mg/week for $\geqslant$12 weeks (at a stable dose for the previous 4 weeks). Active disease was defined as swollen joint count (SJC) $\geqslant$8 (66-joint count) and tender joint count (TJC) $\geqslant$8 (68-joint count) at screening and baseline, with CRP $\geqslant$6 mg/l or ESR $\geqslant$28 mm/h.

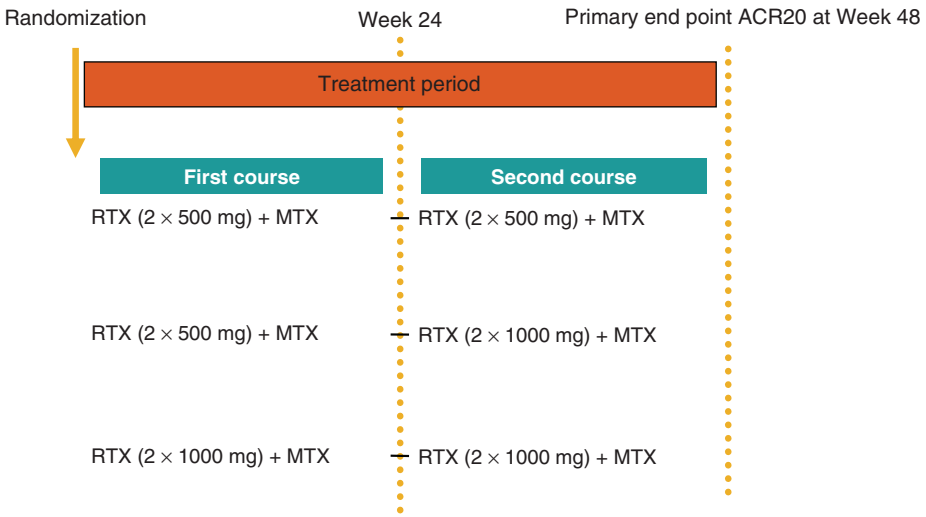
Key exclusion criteria included the earlier receipt of more than one biological agent approved for use in RA; significant systemic involvement secondary to RA; a history of current inflammatory joint disease other than RA or another systemic autoimmune disorder; significant cardiac or pulmonary disease; active infection or history of serious recurrent or chronic infection.

Assessments

The primary endpoint was the proportion of patients with an ACR20 response at Week 48 [8]. Secondary endpoints at Week 48 included ACR50 and ACR70 responses; changes from baseline in disease activity score (DAS-28-ESR) [9]; European League Against Rheumatism (EULAR) response [10]; change from baseline in Medical Outcomes Study Short Form (36-item) Health Survey (SF-36) subscale and summary scores [11, 12]; and Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) assessment [13]. Exploratory endpoints included proportion of patients achieving DAS-28-ESR remission, defined as a DAS-28-ESR <2.6 [9], assessment of function using the HAQ-Disability Index (HAQ-DI) and the proportion of patients with a minimal clinically important difference (MCID) in HAQ-DI, defined as an improvement of at least 0.22 [14].

Pharmacodynamic outcomes included peripheral B-cell and T-cell counts (measured by flow cytometry), immunoglobulin (Ig) concentrations (including isotypes), presence

FIG. 1 Overview of study design.



of human anti-chimeric antibodies (HACAs) and levels of both RF and anti-citrullinated peptide antibodies [by detection of anti-cyclic citrullinated (aCCP) antibodies].

Clinical adverse events (AEs) were recorded throughout the study and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAEs), version 3 [15]. Serious AEs (SAEs) were defined as per the International Conference on Harmonization (ICH) criteria [16].

Statistical analysis

Randomization was stratified by region, RF ($RF^+ \geqslant 20$ IU/ml or $RF^- < 20$ IU/ml) and an earlier biological use. Seronegative patients and patients with earlier exposure to biological therapies were limited to not more than 20 and 30% of the total population, respectively.

A sample size of 125 patients per arm (375 patients in total) was determined to ensure 80% power to discern a 17.5% difference in the proportion of patients with an ACR20 response at Week 48 between the RTX 2×500 mg group and the dose escalation treatment group, using Fisher's exact test with a two-sided significance level of 0.05.

For the primary efficacy variable (ACR20 response at Week 48), the RTX 2×500 mg group was compared with the dose escalation group (the primary analysis) using the Cochran–Mantel–Haenszel (CMH) test and logistic regression analysis, adjusted for baseline factors of RF status, region and earlier biological use. Further exploratory analyses were conducted to compare the 2×500 mg group with the 2×1000 mg group.

Secondary endpoints were analysed to compare the 2×500 mg group with the dose escalation group (the secondary analysis) using the CMH test for categorical endpoints and analysis of variance (ANOVA) for continuous endpoints, both adjusted for baseline stratification factors. ANOVA models also included the baseline value for the endpoint, if applicable. Missing data were imputed using the non-responder method for ACR and EULAR (all patients who withdrew were classed as non-responders); last observation carried forward was used for all other endpoints. Further exploratory analyses were conducted to compare the 2×500 mg group with the 2×1000 mg group.

During the conduct of the study, the sponsor became aware of treatment errors owing to a lack of synchronization between an updated medication list and the randomization schedule. These treatment errors affected 60 patients and subsequently potentially compromised any analysis based on the intent-to-treat population (ITT; all treated patients as randomized). Results are consequently presented from a modified ITT (mITT) analysis with patients analysed by the treatment they actually received as opposed to the treatment they were randomized to receive. Analyses using the standard (as randomized) ITT population were conducted on the primary endpoint (ACR20).

Results

Patient disposition

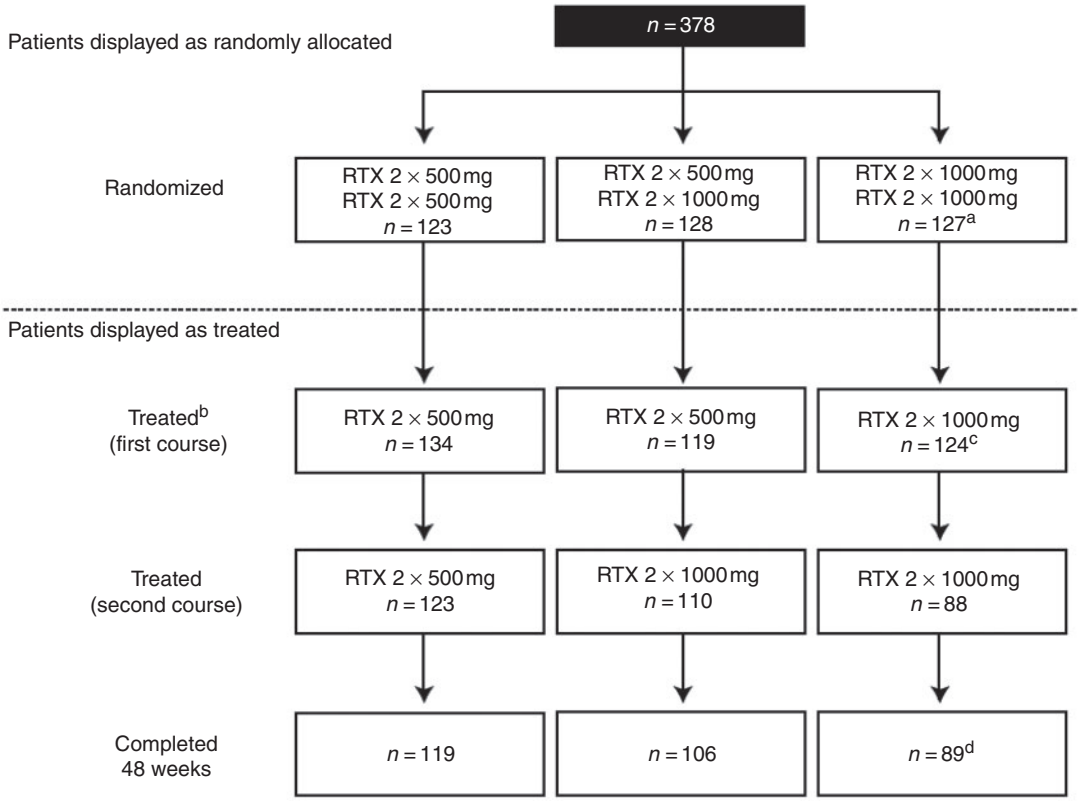
In total, 378 patients were randomly assigned, with all except one receiving at least one infusion. The protocol-defined regimen was given to 346 patients, of whom 314 (83%) completed the full 48-week study (Fig. 2).

Overall, 32 of the 346 patients withdrew before Week 48; the most common reasons for withdrawal being lack of efficacy and withdrawal of consent (10 patients each). Nine (2.6%) patients withdrew due to AEs, which included acute respiratory distress syndrome, bronchopneumonia, hypoxia, myocardial infarction, ovarian epithelial cancer, infusion-related reaction (IRR) and sepsis.

Baseline characteristics and demography

Patient demographic and baseline disease characteristics were well balanced across the three treatment groups

Fig. 2 Disposition of patients up to Week 48. [a]Fourteen patients were randomly assigned to rituximab (RTX) 2 × 1000 mg, placebo. [b]Some patients received a treatment regimen other than that to which they were randomly assigned. [c]Six patients received placebo and 25 patients received RTX 2 × 500 mg for their second course (data on these 31 patients treated with non-protocol-specified regimens not shown). [d]One patient did not receive a second course of treatment, but completed 25 weeks of follow-up.



Reason for withdrawal, *n* (%)			
AE or intercurrent illness	5 (3.7)	3 (2.5)	1 (1.1)
Insufficient therapeutic response	4 (3.0)	4 (3.3)	2 (2.2)
Failure to return	0	1 (0.8)	0
Violation of selection criteria at entry	1 (0.7)	0	1 (1.1)
Withdrew consent	5 (3.7)	5 (4.2)	0
Total	15 (11.2)	13 (10.9)	4 (4.3)

(Table 1), and show that the recruited population had established active disease (baseline DAS-28-ESR ~6.7). Baseline doses of MTX and use of oral corticosteroids were similar across groups (Table 1) with doses remaining stable during the course of the study. Patients had previously been treated with approximately two DMARDs, with ~26% of patients in each group having previously received a TNF inhibitor (Table 1).

Efficacy

At Week 48, ACR20 responses were achieved by 64, 64 and 72% of patients in the RTX 2 × 500 mg, dose escalation and RTX 2 × 1000 mg groups, respectively (Fig. 3), with there being no significant difference in ACR20 response rates between dose groups. ACR50 and ACR70 responses were also similar between the treatment groups. ACR response rates in the RTX 2 × 1000 mg group were somewhat higher than those in both the RTX 2 × 500 mg and dose escalation groups, although the difference was not statistically significant (Fig. 3). Analyses conducted on the primary endpoint using the standard ITT revealed results consistent with the mITT (ACR20 responses were achieved by 64, 65 and 68% of patients in the RTX 2 × 500 mg, dose escalation and RTX 2 × 1000 mg groups, respectively), with there being no significant difference in ACR20 response rates between dose groups [$P = 0.8864$ (dose escalation *vs* 2 × 500 mg); $P = 0.671$ (2 × 1000 *vs* 2 × 500 mg)].

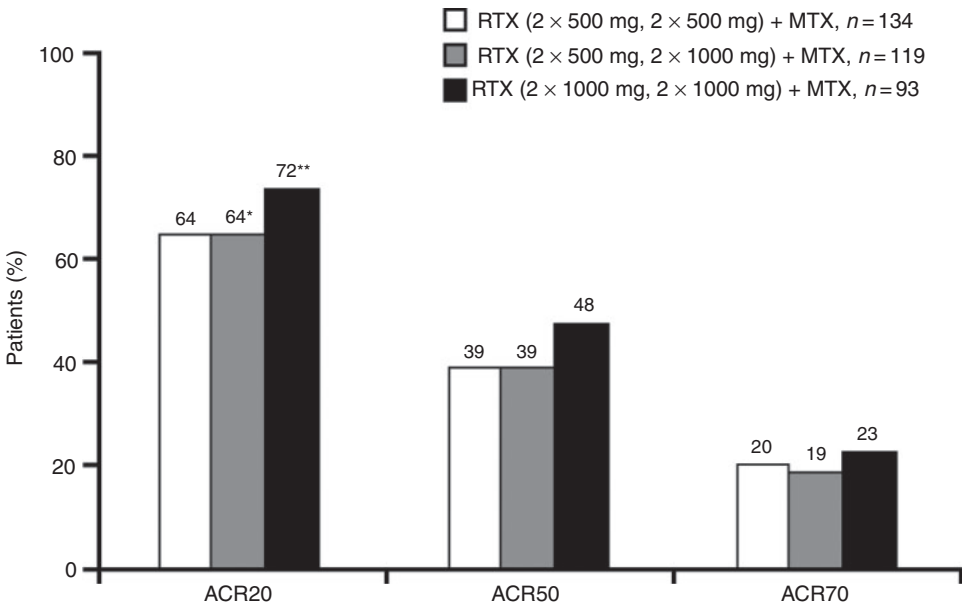
Moderate or good EULAR responses were achieved in 73, 72 and 89% of patients in the RTX 2 × 500 mg, dose

TABLE 1 Patient demographics and baseline disease characteristics

Characteristics	RTX (2 x 500 and 2 x 500 mg) + MTX, *n* = 134	RTX (2 x 500 and 2 x 1000 mg) + MTX, *n* = 119	RTX (2 x 1000 and 2 x 1000 mg) + MTX, *n* = 93
Demographics			
Female, *n* (%)	110 (82.1)	90 (75.6)	77 (82.8)
Age, mean (S.D.), years	53.6 (12.8)	52.3 (12.1)	51.3 (12.2)
Duration of RA, mean (S.D.), years	9.0 (7.4)	9.6 (8.6)	7.7 (7.4)
Previous DMARDs,[a] mean (S.D.), *n*	2.0 (1.5)	2.2 (1.6)	1.8 (1.4)
Earlier TNF inhibitor treatments, *n* (%)	37 (27.6)	31 (26.1)	23 (24.7)
MTX dose, mean (S.D.), mg/week	15.2 (4.7)	15.1[b] (4.5)	15.2 (4.7)
Oral corticosteroid use, *n* (%)	85 (63.4)	78 (65.5)	63 (67.7)
NSAID use, *n* (%)	61 (45.5)	57 (47.9)	52 (55.9)
Disease characteristics			
Mean SJC (66 joints) (S.D.), *n*	18.0 (9.0)	20.3 (10.5)	20.3 (10.5)
Mean TJC (68 joints) (S.D.), *n*	30.9 (13.7)	33.2 (14.1)	33.0 (14.3)
Mean baseline HAQ-DI (S.D.)	1.73 (0.7)	1.74[c] (0.6)	1.61 (0.7)
RF^+, *n* (%)	95 (70.9)	87 (73.1)	64 (68.8)
RF[d], mean (S.D.), IU/ml	235.5 (416.6)	247.7 (416.1)	232.4 (366.1)
ESR, mean (S.D.), mm/h	46.7 (24.2)	47.7 (24.7)	45.2 (28.2)
CRP, mean (S.D.), mg/dl	2.1 (2.4)	2.6 (2.7)	2.2 (2.6)
DAS-28-ESR, mean (S.D.)	6.7[e] (1.0)	6.8 (0.8)	6.7 (0.9)

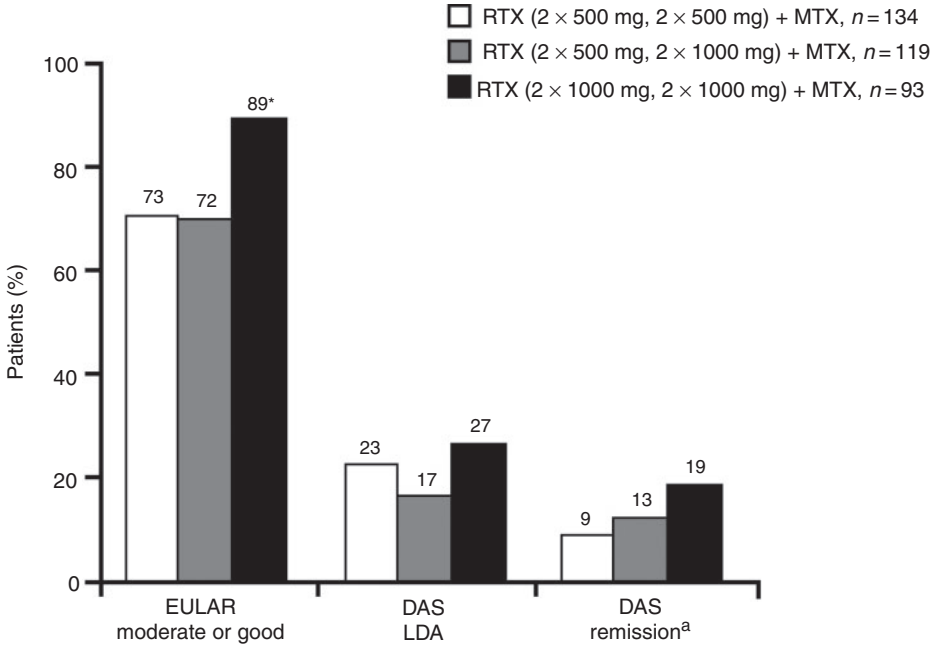
[a]Excludes MTX. [b]$n = 118$. [c]$n = 116$. [d]$\geqslant 20$ IU/ml. [e]$n = 133$.

FIG. 3 Number of patients achieving an improvement in ACR criteria at Week 48 (mITT population). *$P = 0.8156$. **$P = 0.2419$ for RTX (2 × 500 and 2 × 500 mg) *vs* RTX (2 × 500 mg, 2 × 1000 mg) and RTX (2 × 1000 and 2 × 1000 mg), respectively.



escalation and RTX 2 × 1000 mg groups, respectively (Fig. 4). EULAR responses were achieved by significantly more patients in the rituximab 2 × 1000 mg group compared with the RTX 2 × 500 mg group (89 *vs* 73%, $P = 0.0495$). Although no significant differences in DAS remission were observed between treatment groups, numerically higher responses were seen in patients in the RTX 2 × 1000 mg group compared with RTX 2 × 500 mg and dose escalation groups (19 *vs* 9 *vs* 13%, respectively; Fig. 4). Improvement in disease activity, as indicated by a decrease from baseline in mean DAS-28-ESR, was seen and maintained in all groups over the 48-week period (Fig. 5). Following the

FIG. 4 Summary of clinical efficacy at Week 48. [a]DAS-28-ESR <2.6. *$P = 0.0495$ for RTX (2 × 500 and 2 × 500 mg) *vs* RTX (2 × 500 and 2 × 1000 mg). LDA: low disease activity.



second treatment course at Week 24, further improvements in mean DAS-28 were seen in all three treatment groups (Fig. 5).

Mean improvements in the HAQ-DI were observed in all three treatment groups between baseline and Week 48, with no statistically significant differences between the treatment groups (Table 2). Approximately 70% of patients in each of the treatment groups achieved the MCID for HAQ-DI at Week 48.

All three treatment groups showed a similar improvement in mean fatigue score relative to baseline at Week 48 (Table 2). At Week 48, 58, 64 and 69% of patients achieved the MCID for FACIT-F in the RTX 2 × 500 mg, dose escalation and RTX 2 × 1000 mg groups, respectively (Table 2).

All treatments were associated with positive improvements in the mean physical health and mental health component scores of the SF-36, with no statistically significant difference between treatment groups (Table 2). The proportion of patients achieving the MCID for mental component and physical component summary scores at Week 48 was similar between all treatment groups, with higher proportions achieving MCIDs for the physical component summary score (Table 2).

In patients whose ACR*n* was <20 at Week 24 (i.e. ACR20 non-responders), 44, 39 and 46% of patients in the RTX 2 × 500 mg, dose escalation and RTX 2 × 1000 mg groups achieved at least an ACR20 at Week 48 following their respective second treatment courses. Considering patients who had an ACR response at Week 24, 78% of patients receiving RTX 2 × 1000 mg maintained or improved their ACR response compared with 72 and 65% in the dose escalation and RTX 2 × 500 mg groups, respectively. Additionally, fewer patients (22%) receiving RTX 2 × 1000 mg had poorer response at Week 48 compared with 28 and 35% in the dose escalation and RTX 2 × 500 mg groups, respectively (Table 3).

ACR20 response rates at Week 48 were similar in patients who had received an earlier biological treatment (65%) compared with patients who were biological naïve (67%). Similarly, ACR50, ACR70 and EULAR responses at Week 48 were similar, regardless of an earlier biological therapy. Within the earlier biological subgroup, response rates for the RTX 2 × 1000 mg group were consistently higher than those of the RTX 2 × 500 mg group. For example, higher proportions of patients achieved ACR50 (52 *vs* 33%), ACR70 (24 *vs* 18%) and EULAR good or moderate responses (88 *vs* 73%) in the RTX 2 × 1000 mg than the RTX 2 × 500 mg group. However, patient numbers within this subgroup were small and the difference in proportions between treatment groups was not statistically significant.

Pharmacodynamics

Peripheral B-cell levels were fully depleted after the first course of RTX, with no clear difference in peripheral $CD19^+$ B-cell depletion and repletion profiles between the treatment groups over 48 weeks. Median $CD19^+$ B-cell counts of 9–15 cells/μl at 24 weeks and 5–7 cells/μl at 48 weeks were observed. Mean levels of peripheral T cells (CD3) and T-cell subsets ($CD4^+$ and $CD8^+$) remained stable through Week 48 in all three treatment arms, as did memory ($CD3^+$, $CD4^+$ $CD45Ro^+/CD45Ra^-$),

FIG. 5 Plot of mean change from baseline in DAS-28-ESR score by visit, last observation carried forward (LOCF) imputation (mITT population).

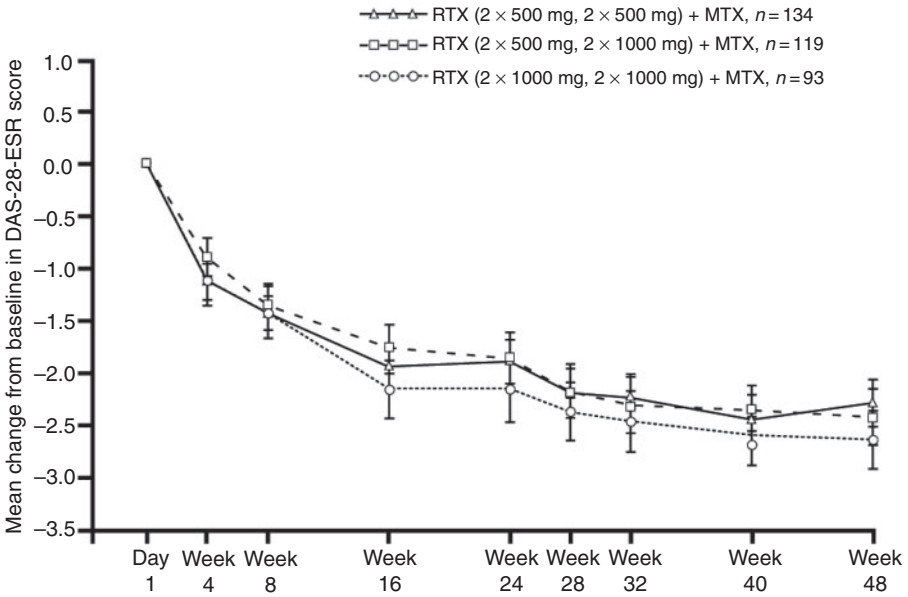


TABLE 2 Summary of patient-reported outcomes at Week 48

Outcomes	RTX (2 x 500 and 2 x 500 mg) + MTX	RTX (2 x 500 and 2 x 1000 mg) + MTX	RTX (2 x 1000 and 2 x 1000 mg) + MTX
HAQ-DI, *n*	134	115	93
Change from baseline score (LOCF), mean (S.D.)[a]	−0.5 (0.6)	−0.6 (0.6)	−0.6 (0.6)
Patients with MCID, *n* (%)[b]	93 (69.4)	86 (72.3)[c]	67 (72.0)
FACIT-F, *n*	125	115	91
Change from baseline (LOCF), mean (S.D.)[d]	6.6 (10.2)	8.1 (10.3)	8.4 (9.8)
Patients with MCID from baseline, *n* (%)[e]	72 (57.6)	74 (64.3)	63 (69.2)
SF-36			
Mean change from baseline (LOCF), *n*	121	112	87
Change in mental health score, mean (S.D.)	5.6 (12.4)	5.0 (11.8)	4.7 (11.1)
Change in physical health score, mean (S.D.)	7.2 (8.5)	7.2 (8.3)	9.0 (9.7)
SF-36			
Mental component summary, *n*	134	118	93
Patients with improved summary score, *n* (%)[f]	58 (43.3)	48 (40.7)	37 (39.8)
SF-36			
Physical component summary, *n*	134	118	93
Patients with improved summary score, *n* (%)[g]	69 (51.5)	66 (55.9)	53 (57.0)

[a]A negative change from baseline indicates an improvement. [b]HAQ-DI score decrease >0.22. [c]*n* = 119. [d]Positive change from baseline indicates an improvement. [e]Change from baseline ⩾4. [f]SF-36 score change >6.33. [g]SF-36 score change >5.42.

naïve ($CD3^+$, $CD4^+$ $CD45Ro^-/CD45Ra^+$) and transitional ($CD3^+$, $CD4^+$ $CD45Ro^+/CD45Ra^+$) subsets.

Following the first treatment course, mean IgA, IgG, IgM and total Ig concentrations declined from baseline levels in all groups, stabilizing between Weeks 8 and 24. Following the second treatment course, mean Ig concentrations underwent a further decline; however, mean concentrations of all isotypes remained within normal limits at all time points up to Week 48. At Week 48, <1% of patients had a total Ig concentration below the lower limit of normal. IgG concentrations were below normal in 1.7, 0 and 0% of patients in the 2 × 500 mg, dose escalation and 2 × 1000 mg groups, respectively. Higher proportions of patients had IgM concentrations below normal limits (13.7, 13.3 and 10.1%, respectively). Levels of RF (including RF isotypes) and aCCP were reduced by ~45% in all three treatment groups by Week 48.

Table 3 Summary of shift in ACR response from Week 24 to Week 48

ACR*n* category at Week 24	Week 24–48 shift in response	RTX (2 x 500 and 2 x 500 mg) + MTX, *n* = 134	RTX (2 x 500 and 2 x 1000 mg) + MTX, *n* = 119	RTX (2 x 1000 and 2 x 1000 mg) + MTX, *n* = 93
<ACR20 (NR)	N	59	44	28
	Improved,[a] *n* (%)	26 (44)	17 (39)	13 (46)
	Remained in NR category, *n* (%)	33 (56)	27 (61)	15 (54)
>ACR20	N	75	74	65
	Improved,[a] *n* (%)	28 (37)	26 (35)	18 (28)
	Maintained,[b] *n* (%)	21 (28)	27 (36)	33 (51)
	Worsened,[c] *n* (%)	26 (35)	21 (28)	14 (22)

[a]Shift upwards by at least one category. [b]Remained in the same category of ACR response. [c]Shift downwards by at least one category. NR: non-response.

At Week 24, the incidence of positive HACA titres was 5.1, 7.3 and 2.3% in the RTX 2 × 500 mg, dose escalation and RTX 2 × 1000 mg groups, respectively, although this declined to 4.3, 1.0 and 2.3%, respectively, by Week 48. In total, 18.8% (3/16) of HACA-positive patients at Week 24 following the first course experienced an IRR during the second exposure to RTX, which is consistent with the overall incidence of IRRs during the second course (17%). The presence of HACAs did not appear to influence either the ability of RTX to deplete $CD19^+$ B cells or efficacy or safety outcomes.

Safety

The incidence of AEs, SAEs and AEs leading to withdrawal (RA flares excluded) was similar across treatment groups (Table 4). Common AEs included RA flares, nasopharyngitis and upper respiratory tract infections and IRRs. IRRs were reported in 39, 30 and 30% of patients in the RTX 2 × 500 mg, dose escalation and RTX 2 × 1000 mg groups, respectively, with the incidence being higher following the first course than following the second course (Table 4). Two patients (both in the RTX 2 × 500 mg group) experienced a serious IRR during the first infusion of the first course, with three further patients experiencing IRRs that were CTC AE Grade 3 events. Multiple symptoms were reported for each IRR and included angioneurotic oedema, bronchospasm, flushing, hypotension, laryngeal or pharyngeal oedema, throat irritation, pruritus and pyrexia.

Approximately 60% of patients experienced at least one infection during the study period. The most frequently reported infections included nasopharyngitis, upper and lower respiratory tract infections (including bronchitis) and urinary tract infections. A total of 11 serious infections were reported, including sepsis, skin ulcer, lower respiratory tract infection and sinusitis in the RTX 2 × 500 mg group; bronchopneumonia, respiratory tract infection, post-operative wound infection, gastroenteritis and bronchitis in the dose escalation group; and diverticulitis and acute pyelonephritis in the RTX 2 × 1000 mg group. The rate of all infections and serious infections per 100 patient-years over 48 weeks was similar across treatment groups (Table 4) and no opportunistic infections were reported during the study period. There was no apparent association between the occurrence of a serious infection and low Ig levels. Indeed, in 9 of 11 cases of serious infection, the patients had Ig concentrations (total and isotype) within the normal range. Two serious infections (bronchitis and diverticulitis) were reported in patients who developed low IgM levels following RTX treatment, although all other isotypes remained within the normal range.

Malignancies were reported in four (1.2%) patients, and included basal cell carcinoma (one case each in RTX 2 × 500 mg and dose escalation groups), squamous cell carcinoma of the skin (dose escalation group) and Hodgkin's disease (RTX 2 × 1000 mg group).

Discussion

The objective of this study was to determine the impact of various repeat treatment regimens with RTX, either at the same dose (two courses of 2 × 500 mg 24 weeks apart) or at a higher dose (dose escalation, 2 × 500 mg followed by retreatment with 2 × 1000 mg). In addition, the standard regimen of two courses of 2 × 1000 mg 24 weeks apart was evaluated. With respect to the primary endpoint (ACR20 at Week 48), there were no statistically significant differences between the three treatment regimens. Although the power of the study to detect dose differences was somewhat compromised by the treatment errors that occurred, analyses based on the ITT population 'as randomized' or 'as treated' (presented in this article), were consistent with each other.

RTX was found to be an effective treatment in patients with an inadequate response to MTX, with some important and relevant clinical observations being made. ACR response rates across the treatment groups at Week 48 were comparable with those previously reported with RTX [1] and also with those reported for biological agents [17–20], albeit with the caveat that in this study there was no control group for comparison. Importantly, high-hurdle disease activity endpoints, such as ACR70, DAS low disease activity or remission at Week 48, were

Table 4 Summary of safety profile over 48 weeks

	RTX (2 x 500 mg, 2 x 500 mg) + MTX, *n* = 134	RTX (2 x 500 mg, 2 x 1000 mg) + MTX, *n* = 119	RTX (2 x 1000 mg, 2 x 1000 mg) + MTX, *n* = 93
Treated first course, *n*	134	119	93
Treated second course, *n*	123	110	88
Patient-years of observation	119.2	105.8	84.8
AEs, *n* (%)			
Any AE	121 (90)	106 (89)	85 (91)
SAE	15 (11)	21 (18)	16 (17)
AE leading to withdrawal[a]	5 (4)	8 (7)	3 (3)
Death	0 (0)	0 (0)	0 (0)
IRR, *n* (%)			
Any IRR	52 (39)	36 (30)	28 (30)
First course			
Any	44 (33)	27 (23)	25 (27)
Serious and/or CTC AE Grade 3	4 (3.0)	0 (0)	0 (0)
Second course[b]			
Any	22 (18)	16 (15)	17 (19)
Serious and/or CTC AE Grade 3	0 (0)	1 (<1)	0 (0)
Malignancy			
Any	1 (<1)	2 (2)	1 (1)
Serious	0 (0)	1 (<1)	1 (1)
Infection			
Any	75 (56)	73 (61)	60 (65)
Serious[c]	4 (3)	4 (3)	2 (2)
Total infections, *n*	144	150	135
Infections per 100 patient-years (95% CI)	120.8 (102.6, 142.2)	141.8 (120.9, 166.4)	159.2 (134.5, 188.4)
Total serious infections, *n*	4	5	2
Serious infections[c] per 100 patient-years (95% CI)	3.4 (1.3, 8.9)	4.7 (2.0, 11.4)	2.4 (0.6, 9.4)

[a]Includes five patients with events of RA flare (primary reason for withdrawal was lack of efficacy and two patients who withdrew for AEs whose day of withdrawal was not available on the database at data cut-off. [b]Percentage based on no treated second course. [c]Reported as serious and/or treated with intravenous antibiotics. GI: gastrointestinal.

achieved by ~20% of patients, further supporting the results of a previous study in patients with active RA resistant to DMARDs [1].

Secondary endpoints broadly supported the primary outcome; however, there was an indication that patients receiving RTX 2×1000 mg in each treatment course achieved better responses. For example, the proportion of patients achieving remission in the RTX 2×1000 mg group was twice that in the RTX 2×500 mg group (9 *vs* 19%, respectively). Similarly, significantly more patients in the RTX 2×1000 mg group achieved a EULAR good or moderate response compared with the RTX 2×500 mg group (89 *vs* 73%, respectively). Supporting these observations, higher proportions of patients in the RTX 2×1000 mg group maintained their Week 24 ACR response category compared with those in the dose escalation and RTX 2×500 mg groups. Indeed, 78% of patients in the RTX 2×1000 mg group who achieved an ACR20 at Week 24 maintained or improved their ACR response category at Week 48. In contrast, 65% of patients initially receiving RTX 2×500 mg maintained or improved their response following a further course of the same lower dose.

The study has also provided insight into the effect of repeat treatment in patients who had not achieved an ACR20 at Week 24. In a recent study, retreatment with RTX in patients who had not achieved a EULAR response on two consecutive visits following an initial course resulted in continued non-response [21], indicating that further treatment of non-responding patients may not be beneficial. However, in this study, of 131 patients across all groups who were ACR non-responders at Week 24, 43% achieved at least an ACR20 response at Week 48 following repeat treatment.

This study is also the first study where a second course of RTX was given at a fixed time interval (24 weeks) following the initial treatment. Repeat treatments in previously reported studies have been given based on clinical symptoms, together with the physicians' decision to give further courses. As a consequence, time intervals between courses were prolonged (~33 weeks), with patients' disease activity returning close to pre-treatment levels before each course [5]. In contrast, the strategy of administering two courses of RTX 24 weeks apart in the current study appeared to induce a sustained decrease in disease activity over time, as illustrated by maintained or improved outcomes in DAS-28 following the 24-week repeat treatment. This fixed repeat treatment approach would, therefore, appear to be more beneficial than

waiting for disease symptoms to flare before offering retreatment. These observations are also supported by recent data indicating that clinical responses were better maintained in patients receiving 24-week treatment courses based on their DAS-28 [22]. Longer term follow-up of both efficacy and safety of rituximab using such repeat treatment regimens is therefore clearly warranted.

The efficacy of rituximab was apparent irrespective of whether patients had received prior treatment with a TNF inhibitor, with patients who had received prior TNF inhibitors deriving as much benefit from RTX treatment as the overall population. Patients in the earlier biological therapy subgroup receiving RTX 2×1000 mg tended to have consistently higher ACR and EULAR outcomes compared with those in the RTX 2×500 mg group. However, these data should be interpreted with caution, as patient numbers in this earlier biological subgroup were small and no statistically significant difference was found between the dose regimens.

The safety profile of rituximab reported in this study was consistent with previous experience, including that of repeated courses, with no new or unexpected safety signals being observed [3–5, 23]. The rates of AEs were similar across treatment groups and were primarily characterized by IRRs and infections, experienced by 34 and 60% of patients, respectively. Clinically significant (serious or CTC AE grade $\geqslant 3$) infusion reactions were uncommon (five events, 3%); however, these led to discontinuation in two patients. Such events were predominately observed in the 2×500 mg dose group and were associated with the first infusion of the first treatment course. The rate of serious infections was lower than that observed in previously published studies (overall 3.36 compared with 4.7–5.2 events per 100 patient-years) [3, 24, 25]. Importantly, there was no association between the incidence of serious infection and the presence of low Ig (including Ig isotypes). Other events of interest included malignancies, the incidence of which was also comparable with that reported in 1039 RA patients treated with RTX [5].

In conclusion, these data support RTX as an effective and well-tolerated therapy in patients with RA and an inadequate response to DMARDs, irrespective of the earlier treatment with a TNF inhibitor. Although RTX doses and retreatment regimens could not be clearly differentiated, several efficacy outcomes favoured treatment with RTX 2×1000 mg. Repeat treatment at Week 24 with RTX maintained the response achieved with the first course and may be associated with improved efficacy outcomes. The safety profile of RTX remained favourable, with no new safety signals becoming apparent with repeat courses.

Rheumatology key messages

- RTX is effective and well tolerated in patients with an inadequate response to DMARDs.
- Some efficacy outcomes suggest improved outcomes for RTX 2×1000 *vs* 2×500 mg.

Acknowledgements

The authors wish to thank the principal investigators of the MIRROR Trial Group. They are also grateful to Jatinderpal Kalsi, Mark Reynard, Helen Tyrrell, Fiona Langley, Jane Baker (Roche Products Ltd) and David Yocum and Nicole Li (Genentech). This manuscript was prepared by Matthew Pugh (Adelphi Communication Ltd) in consultation with Roche, Genentech and the authors.

Funding: This study was sponsored by F. Hoffmann-La Roche, Biogen Idec and Genentech. Funding to pay the Open Access publication charges for this article was provided by F. Hoffmann-La Roche.

Disclosure statement: T.M.S. is an employee of Roche Products Ltd and holds stock in that company. L.C. has received research grant/support from Roche Farma S.A. N.C. and G.A. are employees of Roche Products Ltd. P.P.T. has received a research grant and consulting fees from Roche. A.R.-R. has received consulting fees from Roche, Wyeth, Abbott, UCB, Essex and Bristol Myers Squibb. C.Z. is a member of Speakers Bureau for Novartis, Lilly and Sanofi-Aventis and has received research grants support from Pfizer, MSD, Lilly, Amgen, Novartis and Roche. The other author has declared no conflicts of interest.

References

1 Edwards JC, Szczepański L, Szechiński J *et al*. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004;350:2572–81.

2 Smolen JS, Keystone EC, Emery P *et al*. Consensus statement on the use of rituximab in patients with rheumatoid arthritis. Ann Rheum Dis 2007;66:143–50.

3 Cohen SB, Emery P, Greenwald MW *et al*. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, Phase III trial evaluating primary efficacy and safety at twenty-four weeks. Arthritis Rheum 2006;54:2793–806.

4 Emery P, Fleischmann R, Filipowicz-Sosnowska A *et al*. The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. Arthritis Rheum 2006;54:1390–400.

5 Keystone E, Fleischmann R, Emery P *et al*. Safety and efficacy of additional courses of rituximab in patients with active rheumatoid arthritis. Arthritis Rheum 2007;56:3896–908.

6 Breedveld F, Agarwal S, Yin M *et al*. Rituximab pharmacokinetics in patients with rheumatoid arthritis: B-cell levels do not correlate with clinical response. J Clin Pharmaco 2007;47:1119–28.

7 Dass S, Rawstron AC, Vital EM *et al*. Highly sensitive B cell analysis predicts response to rituximab therapy in rheumatoid arthritis. Arthritis Rheum 2008;58:2993–9.

8 Felson DT, Anderson JJ, Boers M *et al*. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727–35.

9 Prevoo ML, Aarden L, Kuper HH *et al*. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.

10 van Gestel AM, Prevoo ML, van't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European league against rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996;39:34–40.

11 Lubeck DP. Patient-reported outcomes and their role in the assessment of rheumatoid arthritis. Pharmacoeconomics 2004;22:27–38.

12 Norman GR, Sloan JA, Wyrwich KW. Interpretation of changes in health-related quality of life: the remarkable universality of half a standard deviation. Med Care 2003; 41:582–92.

13 Cella D, Yount S, Sorensen M *et al*. Validation of the functional assessment of chronic illness therapy fatigue scale relative to other instrumentation in patients with rheumatoid arthritis. J Rheumatol 2005;32:811–9.

14 Wells GA, Tugwell P, Kraag GR, Baker PR, Groh J, Redelmeier DA. Minimum important difference between patients with rheumatoid arthritis: the patient's perspective. J Rheumatol 1993;20:557–60.

15 CTC Cancer Therapy Evaluation Program, common terminology criteria for adverse events. version 3.0. http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm (28 September 2009, last date accessed).

16 International Conference on Harmonisation Criteria: safety pharmacology studies for human pharmaceuticals. Step 4 version. http://www.ich.org/LOB/media/MEDIA504.pdf (28 September 2009, last date accessed).

17 Kremer JM, Dougados M, Emery P *et al*. Treatment of rheumatoid arthritis with the selective costimulation modulator abatacept: twelve-month results of a Phase IIb, double-blind, randomized, placebo-controlled trial. Arthritis Rheum 2005;52:2263–71.

18 Maini R, St Clair EW, Breedveld F *et al*. Infliximab (chimeric anti-tumour necrosis factor a monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999;354:1932–9.

19 Weinblatt ME, Keystone EC, Furst DE *et al*. Adalimumab, a fully human anti-tumor necrosis factor α monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003;48:35–45.

20 Kavanaugh A, Klareskog L, van der HD, Li J, Freundlich B, Hooper M. Improvements in clinical response between 12 and 24 weeks in patients with rheumatoid arthritis on etanercept therapy with or without methotrexate. Ann Rheum Dis 2008;67:1444–7.

21 Thurlings RM, Vos K, Gerlag DM, Tak PP. Disease activity-guided rituximab therapy in rheumatoid arthritis: the effects of re-treatment in initial nonresponders versus initial responders. Arthritis Rheum 2008;58: 3657–64.

22 Emery P, Mease P, Rubbert-Roth A *et al*. Retreatment with rituximab based on a treatment to target (TT) approach provides better disease control than treatment as needed (PRN) in patients with rheumatoid arthritis. Arthritis Rheum 2009;60(Suppl. 10):S753.

23 Emery P, Rigby WF, Combe B *et al*. Efficacy and safety of rituximab (RTX) as first-line biologic therapy in patients (pts) with active rheumatoid arthritis (RA): results of a Phase III randomized controlled study (SERENE). Abstract No. 364. 2008 American College of Rheumatology Scientific Meeting, San Francisco, USA 24–29 October 2008. Arthritis Rheum 2008;58(Suppl. 9):S302.

24 Genovese M, Emery P, Ruderman E *et al*. Immunoglobulin levels and infection rates in patients with rheumatoid arthritis treated with repeated courses of rituximab. Arthritis Rheum 2007;56(Suppl. 9):S149.

25 Gottenberg JE, Ravaud P, Bardin T *et al*. Prospective follow-up of rituximab treatment in 965 patients with refractory rheumatoid arthritis (630 patients/year): tolerance and efficacy data from the French registry AIR (Autoimmunity And Rituximab). Abstract No.1190. 2008 ACR, San Francisco, CA, USA. Arthritis Rheum 2008;58(Suppl. 9):S611.

Appendix 1
MIRROR Trial Group investigators

Australia: Dr P. Nash, Dr H. Bagga, Prof. S. Hall, Prof. G. Littlejohn and Prof. N. Manolios; Belgium: Dr F. Van Den Bosch; Brazil: Dr A. C. Ximenes; Canada: Dr T. Mccarthy, Dr C. Atkins, Dr J. C. Thorne, Dr R. Offer, Dr J. Pope and Dr E. Keystone; China: Prof. F. Huang and Prof. D. Wu; Finland: Dr A. Karjalainen; France: Prof. T. Schaeverbeke, Prof. L. Euller-Ziegler, Prof. C. Jorgensen, Dr M. Nguyen and Dr X. Puechal; Germany: Dr S. Wassenberg, Dr B. Heilig, Prof. U. Mueller-Ladner, Prof. J. Wollenhaupt, Dr J. Braun and Dr G. Gauler; Hungary: Dr Z. Szekanecz and Dr K. Nagy; Italy: Dr M. Galeazzi, Prof. F. Trotta, Prof. S. Bombardieri, Dr C. Salvarani, Dr R. Pellerito, Prof. B. Seriolo, Prof. S. Adami, Prof. G. Bianchi, Prof. R. Giacomelli, Prof. G. Arioli and Prof. F. Cantini; New Zealand: Dr D. Ching, Dr N. Gilchrist and Dr J. Singh; Slovakia: Dr J. Lukac, Dr J. Rovensky and Dr Z. Macejova; South Africa: Prof. A. Kalla, Dr M. Tikly, and Dr M. M. T. Ally; Spain: Dr J. L. Marenco, Dr T. Tinture, Dr J. Juanola, Dr J. M. Alvaro-Gracia, Dr J. M. Rodriguez-Heredia and Dr T. P. Sandoval; Taiwan: Dr T. T. Cheng, Dr S. F. Luo, Dr J. L. Lan, Dr M. Osiri, Dr S. Nilganuwong, Dr P. Asavatanabodee, Dr R. Nanagara and Prof. W. Louthrenoo; UK: Dr B. L. Hazleman, Dr R. Jubb, Dr E. George, Dr A. Hakim, Dr D. Grennan, Dr P. Gardiner, Dr J. Taylor, Dr J. Mcnally, Dr D. Hutchinson and Dr M. Plant.